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# Antidiabetic Activity of the Alcoholic Extract of the Aerial Part of *Boerhaavia diffusa* in Rats

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Article Info	Abstract
<b>Article History</b> Received : 21/02/2011 Revised : 26/03/2011 Accepted : 26/03/2011	This study indicates that <i>Boerhaavia diffusa</i> alcoholic extracts have good antidiabetic activity. Methanolic and ethanolic extracts of <i>Boerhaavia diffusa</i> at a dose level of 200 mg/kg/p.o exhibited significant anti-hyperglycemic activities in alloxan induced as well as in streptozotocin induced hyperglycemic rats. They can also improve the condition of diabetes as indicated by parameters like body weight along with serum cholesterol and triglyceride levels. The extracts of the plant <i>Boerhaavia diffusa</i> was tested for oral hypoglycaemic and anti-diabetic activity, by glucose oral tolerance test, alloxan induced and streptozotocin induced diabetic rats respectively. The extracts of <i>Boerhaavia diffusa</i> have shown significant (P<0.001) increase in glucose tolerance, but methanolic extract shows more activity. In alloxan-induced diabetic rats also the maximum percentage reduction in blood glucose levels was found to be in <i>Boerhaavia diffusa</i> methanolic extract. Animals, which received STZ, also showed a significant reduction in body weight, and increase in water and food intake as compared to vehicle control, which is significantly reversed by methanolic extracts of <i>Boerhaavia diffusa</i> and after 21 days of treatment. These results indicate that the plant <i>Boerhaavia diffusa</i> possess significant anti – diabetic activity
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## Introduction

Diabetes Mellitus is the name given to a group of disorders characterised by chronic hyperglycemia, polyuria, polydipsia, polyphagia, emaciation, and weakness due to disturbance in carbohydrate, fat, and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action. [1] In the recent past many hypoglycaemic agents are introduced, still diabetes and related complications continue to be a major medical problem not only in developed countries but also in developing countries. Many Indian medicinal plants are reported to be useful in diabetes. [2, 3]

*Boerhaavia diffusa* belongs to the family Nyctagenaceae. Its leaves are used as vegetables; the root juice is used to cure asthma, urinary disorders, rheumatism, liver disorders and diabetes. It has a long history of uses by indigenous and tribal people and in Ayurvedic and Natural Herbal Medicine. [4, 5]

Hepatoprotective activity of aqueous extract of roots of *Boerhaavia diffusa* in thioacetamide intoxicated rats.[6] immunomodulatory effects of *Boerhaavia diffusa* alkaloidal fraction reactions in mice,[7] the anti-lymphoproliferative activity of ethanolic extract of *Boerhaavia diffusa* roots against mouse macrophage cell,[8] Immunomodulation activity of ethanolic extract of *Boerhaavia diffusa* roots by inhibited human NK cell cytotoxicity in vitro and production of NO in mouse macrophage cell,[9] chemopreventive action of leave extract of *Boerhaavia diffusa* on 7, 12-dimethyl benza

anthracene induced skin papillomagenesis in mice,[10] inhibitory effect of aqueous methanol (3:7) extract of roots of *Boerhaavia diffusa* was evaluated on experimental metastasis by B16 F10 melanoma in C57BL/6 mice,[11] the immunosuppressive properties of flavonoids isolated from the *Boerhaavia diffusa* hexane chloroform and ethanol root extract,[12] antibacterial activity of ethanol extract of leaves of *Boerhaavia diffusa* against *Bacillus subtilis* and *Escherichia coli* [13]and anti-inflammatory activity of ethanolic and aqueous extract in histamine induced rat paw models.[14] The present study was undertaken to verify the claim and evaluate the anti-diabetic and hypoglycemic activity of the plant *Boerhaavia diffusa*.

## Experimental

### Plant Material

The aerial part of *Boerhaavia diffusa* were collected from the local areas around the jaipur, India, respectively and after authentication by botanist, voucher specimens are being maintained in the laboratory of Phytochemistry and Pharmacognosy, NIMS Institute of Pharmacy, Shobha Nagar, Jaipur, India (voucher specimen no.NIMS/2010/VBBD). The aerial part of whole plant of *Boerhaavia diffusa* were shade dried and chopped into small pieces.

### Preparation of extracts

The aerial part of plant (shade dried) of *Boerhaavia diffusa* were powdered (500g) and extracted with ethanol and methanol in two Soxhlet extractors separately exhaustively for 20-24 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using flash evaporator.

### Test animals

Male wistar albino rats (160 –200g) were used in the experiment. Animals maintained under standard environmental conditions, were fed with a standard diet (Hindustan Lever, India) and water ad libitum. The animals were fasted for 16 h before experimentation but allowed free access to water. Institutional animal Ethics Committee's permission was obtained before starting the experiments on animal.

### Effect of *Boerhaavia diffusa* extracts on oral glucose tolerance in rats

Male wistar albino rats (160 – 200 g), fasted for 16h before experimentation but allowed free access to water and were divided into 3 groups of 6 rats each. Group I control and received distilled water, Group II received ethanolic extract of plant *Boerhaavia diffusa* aqueous suspension orally. Group III received methanolic extract of plant *Boerhaavia diffusa* aqueous suspension orally. All groups were given glucose (2 g/kg body weight, p.o) 30 min after administration of the drug. Blood samples were collected from the tail vein just prior to glucose administration and at 30 and 90 minutes after the glucose loading. Serum was separated and glucose levels were measured immediately by glucose-oxidase method. [15]

### Effect of the *Boerhaavia diffusa* extracts on alloxan-induced diabetic rats

Male wistar rats (160-200g) were made diabetic by a single i.p. injection of 120mg/kg body weight of alloxan monohydrate in sterile normal saline (Insulin dependent diabetes mellitus) [16]. The rats were maintained on 5 % glucose solution for next 24 h to prevent hypoglycaemia. Five days later blood samples were drawn from tail vein and glucose levels were determined to confirm the development of diabetes.

The diabetic rats were divided into four groups, each containing six animals. Controls rats (Group I) were given distilled water orally, *Boerhaavia diffusa* ethanol extracts were given to groups I, *Boerhaavia diffusa* methanol extract was given to groups III, at a dose of 200 mg/kg, orally. Group IV received glibenclamide at dose of 10 mg/kg. Blood samples were collected from the tail vein just prior to and of 1h, 3h and 5h after drug administration [17, 18].

### Effect of the *Boerhaavia diffusa* extracts on streptozotocin induced diabetes

Sprague Dawley rats were used, to induce Non-insulin dependent diabetes mellitus, a single dose of injection of streptozotocin (90 mg/kg: i.p.) was given to the 2 days old pups. Another group of pups received only saline. The animals were weaned at 30 days and after a period of 3 months; they were checked for fasting glucose levels to confirm the status of NIDDM. The animals showing fasting glucose levels > 140 mg/dl were considered as diabetic. The pups that received saline were considered as control animals.

The experimental animals were divided in five groups, six animals in each group. Group I vehicle control, Group II, and Group III NIDDM treated with *Boerhaavia diffusa* ethanolic extract and ethanolic extract 200 mg/kg respectively, and group IV treated with standard drug glibenclamide (10 mg/kg per day p.o), Treatment was given daily for 3 weeks. At the end of 3 weeks treatment, the animals were kept on 12 h fasting and the blood samples were collected from the tail vein. Serum samples were analysed spectrophotometrically for glucose, cholesterol and triglycerides. Body weight, Water intake (ml/rat/day) and food intake (grams/rat/day) were also recorded. [19, 20]

### Statistical analysis

The results are expressed as mean  $\pm$  S.E.M. the significant of various treatments was calculated using students t-test.

### Results

The extracts of *Boerhaavia diffusa* and have shown significant ( $P < 0.001$ ) increase in glucose tolerance (Table 1). Both the extracts reduced the glucose levels approximately near to normal within 90 minutes of the drug administration but the maximum effect was given by ethanolic extract of the plant *Boerhaavia diffusa*.

In alloxan-induced diabetic rats also, both extracts have shown considerable reduction in blood glucose levels. The maximum percentage reduction in blood glucose levels was found to be in ethanolic extract of plant *Boerhaavia diffusa*. The results are graphically represented in Table 2.

Administration of the STZ (90 mg/kg: i.p.) led to significantly elevated levels of Serum glucose, cholesterol and triglycerides in experimental animals in comparison to vehicle control. Treatment with *Boerhaavia diffusa* methanolic extracts and standard drug has shown significant reduction in levels of these parameters as compared to diabetic control rats (Table 3).

Animals, which received STZ, also showed a significant reduction in body weight, and increase in water and food intake as compared to vehicle control, which is significantly reversed by alcoholic extracts of *Boerhaavia diffusa* and after 21 days of treatment (Table-4).

Table 1: Effect of *Boerhaavia diffusa* and extracts on oral glucose tolerance in rats

Group	Treatment (dose/kg body weight)	Blood glucose (mg/dl)		
		Fasting	30 minutes	90 minutes
I	Glucose; 2g	79.25 ± 0.079	148.90 ± 1.96	116.71 ± 1.80
II	BDEE 200 mg + Glucose	78.19 ± 1.15	99.84 ± 0.80*	86.17 ± 0.98*
III	BDME 200 mg + Glucose	79.34 ± 0.96	95.16 ± 1.71*	83.24 ± 1.21*

Values are means ± S.E.M., n = 6,

\* P<0.001 vs group I, *L. aspera* and *P. bigeminum* extracts were given orally 30 min before glucose loading to their respective groups

Table 2: Effect of various extracts of *Boerhaavia diffusa* in Alloxan induced diabetic albino rats

Group	Treatment	Blood glucose at different hours after the treatment			
		0 hr.	1 hr.	3 hr.	5 hr.
I	Diabetic untreated	387.42 ± 6.7 5	382.87 ± 7.21	383.21 ± 7.57	377.21 ± 6.48
II	Diabetic treated with BDEE 200 mg	377.21 ± 7.6 5	292.34 ± 6.21**	286.18 ± 5.78**	289.14 ± 7.82**
III	Diabetic treated with BDME 200 mg	380.21 ± 8.7 5	280.76 ± 8.17**	266.81 ± 4.76**	272.45 ± 7.82**
IV	Diabetic treated with 10 mg/kg of glibenclamide	387.24 ± 6.7 5	264.32 ± 5.33*	253.44 ± 7.04**	222.18 ± 5.48**

Values are means ± S.E.M., n = 6,

\* P<0.001 vs group I, *L. aspera* and *P. bigeminum* extracts were given orally 30 min before glucose loading to their respective groups

Table 3: Effect of extracts of *Boerhaavia diffusa* and on serum profile in diabetic rats (STZ) after treatment of 21 days

Group	Treatment	S. glucose	S. cholesterol	S. triglycerides
I	Vehicle control	75.23 ± 1.71	48.81 ± 2.21	50.36 ± 4.15
II	Diabetic untreated	191.12 ± 6.81*	81.13 ± 1.15*	126.43 ± 7.1*
III	Diabetic treated with BDEE 200 mg	121.17 ± 0.48**	60.16 ± 0.64**	68.52 ± 6.31**
IV	Diabetic treated with BDME 200 mg	114.3 ± 4.71**	58.38 ± 0.94**	62.19 ± 6.19**
V	Diabetic treated with 10 mg/kg of glibenclamide	96.62 ± 1.73**	53.31 ± 1.91**	56.07 ± 5.19**

Each value is mean ± SEM (n=6).

\*significantly different from vehicle control (P < 0.05) \*\*significantly different from diabetic untreated (P < 0.05)

Table 4: Effect of various extracts of *Boerhaavia diffusa* in streptozotocin induced diabetic albino rats after 21 days of treatment

Group	Treatment	Average Body weight (grams/rat)	Water intake (ml/rat/day)	Food intake (grams/rat/day)
I	Vehicle control	206.12 ± 2.91	39.71 ± 1.82	18.57 ± 1.52
II	Diabetic untreated	153.36 ± 2.48*	52.25 ± 1.91*	35.38 ± 1.17*
III	Diabetic treated with BDEE 200 mg	180.42 ± 2.67**	43.13 ± 1.25**	22.14 ± 1.26**
IV	Diabetic rats treated with BDME 200 mg	184.3 ± 4.71**	41.02 ± 1.93**	23.43 ± 1.21**
VI	Diabetic rats treated with 10 mg/kg of glibenclamide	196.42 ± 2.73**	40.13 ± 2.1**	20.31 ± 2.14**

Each value is mean ± SEM (n=6). \*significantly different from vehicle control (P < 0.05), \*\*significantly different from diabetic untreated (P < 0.05) BDEE:- Ethanollic extract of arial parts of *Boerhaavia diffusa* and BDME:- Methanolic extract of arial parts of plant *Boerhaavia diffusa*

## Discussion

This study indicates that *Boerhaavia diffusa* and ethanolic extracts exhibit significant anti-hyperglycemic activities in alloxan induced as well as streptozotocin induced hyperglycemic rats. They can also improve the condition of diabetes as indicated by parameters like body weight along with serum cholesterol and triglyceride levels.

The number of functionally intact β-cells in the islet organ is of decisive importance for the development course and

outcome of diabetes. The renewal of β-cells in diabetes has been studied in several animal models. The total β-cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet β-cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug [21]. In alloxan-induced diabetes, (-)-Epicatechin [22] and *Vinca rosea* extracts [23] has also been shown to act by β-cells regeneration. Similar effects in streptozotocin-treated

diabetic animals were reported by pancreas tonic [24], ephedrine [25], and *Gymnema sylvestre* leaf extracts.[26] In the current studies, the damage of pancreas in streptozotocin-treated diabetic control rats and regeneration of  $\beta$ -cells by glibenclamide was observed. The comparable regeneration was also shown by methanolic extracts of *Boerhaavia diffusa*.

#### Conclusion

The data obtained from this study indicates that the ethanolic extracts of the plant *Boerhaavia diffusa* and are capable of exhibiting significant antihyperglycemic activities in diabetic rats. The extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of  $\beta$ -cells of pancreas and so might be valuable in diabetes treatment.

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